TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35.U.S.C. 371

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09/786024

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/CN99/00132	30 AUGUST 1999	31 AUGUST 1998

TITLE OF INVENTION
A NOVEL HUMAN LYSOZYME GENE, ITS ENCODED POLYPEPTIDE AND THE METHOD FOR PREPARING THEM

APPLICANT(S) FOR DO/EO/US
Long Yu, Qiang Fu, Yong Zhao, Honglai Zhang and Anding Bi

Applicant herewith submits to the United States Designated /Elected Office (DO/EO/US) the following items and other information:

1. [x] This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. [x] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [ ] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [ ] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [x] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [x] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [x] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [x] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [x] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [ ] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [x] Other items or information:

    Verified Statement claiming small entity status
    First page of WO00/12723
    Copy of International Application as published
    (incl. 2 sheets drawings, 1 page claims, 14 pages spec)
    International Search Report
    International Preliminary Exam
    Translation of Priority document

INTERNATIONAL APPLICATION NO. PCT/CN00/00224	INTERNATIONAL FILING DATE 30 AUGUST1999	PRIORITY DATE CLAIMED 31 AUGUST 1998

17. [X] The following fees are submitted:

CALCULATIONS PTO USE ONLY

Basic National Fee (37 CFR 1.492(a)(1)-(5):

Neither international preliminary examination fee (37 CFR 1.482)

Nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO (1.492(a)(3)) $1,000.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO (1.492(a)(5) $860.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO(1.492(a)(2)) $710.00

International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) (1.492(a)(1)) $690.00

International preliminary examination fee paid to USPTO (37 CFR 1 482) and all claims satisfied provisions of PCT Article 33(1)-(4) ..... $ 100.00

ENTER APPROPRIATE BASIC FEE AMOUNT =	$1,000.00	
Surcharge of $130.00 for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 C.F.R. 1.492)(e)).	$	

Claims	Number Filed	Number Extra	Rate	$	
Total Claims	14 -20=		X $ 18.00	$	
Independent Claims	3 -3=		X $ 80.00	$	
Multiple dependent claim(s) (if applicable)			+ $270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$1,000.00	
Reduction by ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$ 500.00	
SUBTOTAL =				$ 500.00	
Processing fee of $130.00 for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).			+	$	
TOTAL NATIONAL FEE =				$ 500.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property			+	$ 40.00	
TOTAL FEES ENCLOSED =				$ 540.00	
				Amt. refunded	$
				charged	$

a. [X] A check in the amount of $ 500 & 40 to cover the above fees is enclosed.

b. [ ] Please charge our Deposit Account No. 02-4377 in amount of $___ to cover the above fees. A copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4377. A copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

BAKER BOTTS L.L.P.
30 Rockefeller Plaza
New York, New York 10112-4498

Signature RONALD B. HILDRETH

February 28, 2001
Date

19,498
Registration No.

FILE NO.A34054-PCT-USA 072975.0110
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Yu et al.		
Serial No.	:	09/786,024	Examiner :	TBA
Filed	:	August 30, 1999	Group Art Unit:	TBA
For	:	A NOVEL HUMAN LYSOZYME GENE, ITS ENCODED POLYPEPTIDE AND THE METHOD OF PREPARING THEM		

PRELIMINARY AMENDMENT AND SUBMISSION OF SEQUENCE LISTING

I hereby certify that this paper is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231

July 2, 2001
Date of Deposit

Rochelle K. Seide
Attorney Name

32,300
PTO Registration No

Rochelle K. Seide
Signature

July 2, 2001
Date of Signature

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In response to the Notification of Missing Requirements mailed on April 30, 2001, please consider the following amendments and remarks. Applicants submit herewith a

Sequence Listing in computer and paper form.

IN THE SPECIFICATION:

Please **amend** the paragraph beginning at page 6, line 24, and ending at page 6, line 29 with the following rewritten paragraph:

In one embodiment, the polynucleotide of the invention is 544 bp in full length whose detailed sequence is shown in SEQ ID NO: 3 with the ORF located at positions 81-521. Said polynucleotide was obtained as follows: human brain gt 11 cDNA library (Clontech) was used as a template and PCR was carried out with the synthetic forward primer A1 5'-AGAGTGGTGGTGGCTCCACTCTG-3' (SEQ ID NO. 1) and reverse primer B :5'-TGCTGTGCATGGTTCCGTCCATC-3' (SEQ ID NO. 2). A target fragment of 544bp was obtained. The sequencing of the PCR product gave the full length cDNA sequence shown in SEQ ID NO: 3.

Please **amend** the paragraph beginning at page 7, line 8, and ending at page 7, line 13 with the following rewritten paragraph:

Fig. 1 shows an alignment comparison of amino acid sequences of human LYC3 and other lysozymes. Fig. 1A shows a homology comparison of amino acid sequences of human LYC3 (SEQ ID NO. 4) and lysozyme C of *Trachypithecus francoisi* (gi|1790947)(SEQ ID NO. 11). Fig. 1B shows a homology comparison of amino acid sequences of human LYC3 (SEQ ID NO. 4) and lysozyme C of ring-necked pheasant (sp|p00702)(SEQ ID NO. 12). The identical amino acids are indicated by ":" between the sequences, and the similar amino acids indicated by ".". The similar amino acids are as follows: A,S,T; D,E; N,Q; R,K; I,L,M,V; F,Y,W.

Please **amend** the paragraph beginning at page 8, line 14, and ending at page 8, line 20 with the following rewritten paragraph:

In particular, in amino acid sequence of LYC3, there exists a 19 amino acids signature sequence of lysozyme and alpha-lactoalbumin: **$CX_3CX_2(L/M/F)X_3(D/E/N)(L/I)X_5C$** (SEQ ID NO. 10) [Note: In the sequence, X represents any amino acid, digits such as "2" denote the number of amino acid, "(L/M/H)" represents any of these three amino acids]. Lysozyme and alpha-lactoalbumin are two proteins related closely in evolution (Eur. J. Biochem. 182: 111-118). In the protein of the present invention, the sequence matching the signature is: CRMYCSDLLNPNLKDTVIC (residues 93-111 in SEQ ID NO: 4). It indicates that the LYC3 of the present invention belongs to lysozyme family, and has the relative functions of the lysozyme family.

Please **delete** the Sequence Listing beginning at page 13, line 1 and ending at page 14, line 53 and **substitute** therefor, the Sequence Listing included herewith.

REMARKS

The Notification of Missing Requirements mailed on April 30, 2001 for the above-identified application alleges that the application fails to comply with the requirements of 37 C.F.R. 1.821 through 1.825. Applicants submit herewith a Sequence Listing in computer and paper form.

A Sequence Listing in computer readable format has not previously been filed in this application. Applicants submit herewith an initial copy of the Sequence Listing in computer form and a substitute copy of the Sequence Listing in paper form.

Rewritten paragraphs appear in the preceding "IN THE SPECIFICATION" section. Attached hereto is a marked-up version of the changes made to the specification paragraphs by the instant amendment. The attached pages are captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE" and are only included for the Examiner's convenience. Should any discrepancies be discovered, the version presented in the preceding "IN THE SPECIFICATION" section shall take precedence.

The content of the paper and computer readable copies of the Sequence Listing submitted in accordance with 37 C.F.R. §1.821(c) and (e) are the same and do not include new matter.

Applicants do not believe that any fee is due with this submission. However, please charge any fees associated with this filing or credit any overpayment to Deposit Account No. 02-4377. Two copies of this paper are enclosed.

Applicants enclose a copy of the Notification of Missing Requirements.

Respectfully submitted,

Ronald B. Hildreth
PTO Reg. No. 19,498

Rochelle K. Seide
PTO Reg. No. 32,300
Attorneys for Applicant

BAKER BOTTS, L.L.P.
30 Rockefeller Plaza
New York, NY 10112
(212) 408-2626

VERSION WITH MARKINGS TO SHOW CHANGES MADE

The paragraph beginning at page 7, line 8, and ending at page 7, line 13 has been amended as follows:

In one embodiment, the polynucleotide of the invention is 544 bp in full length whose detailed sequence is shown in SEQ ID NO: 3 with the ORF located at positions 81-521. Said polynucleotide was obtained as follows: human brain gt 11 cDNA library (Clontech) was used as a template and PCR was carried out with the synthetic forward primer A1 5'-AGAGTGGTGGTGGCTCCACTCTG-3' (SEQ ID NO. 1) and reverse primer B :5'-TGCTGTGCATGGTTCCGTCCATC-3' (SEQ ID NO. 2). A target fragment of 544bp was obtained. The sequencing of the PCR product gave the full length cDNA sequence shown in SEQ ID NO: 3.

The paragraph beginning at page 7, line 8, and ending at page 7, line 13 has been amended as follows:

Fig. 1 shows an alignment comparison of amino acid sequences of human LYC3 and other lysozymes. Fig. 1A shows a homology comparison of amino acid sequences of human LYC3 (SEQ ID NO. 4) and lysozyme C of *Trachypithecus francoisi* (gi|1790947)(SEQ ID NO. 11). Fig. 1B shows a homology comparison of amino acid sequences of human LYC3 (SEQ ID NO. 4) and lysozyme C of ring-necked pheasant (sp|p00702)(SEQ ID NO. 12). The identical amino acids are indicated by ":"

between the sequences, and the similar amino acids indicated by ":". The similar amino acids are as follows: A,S,T; D,E; N,Q; R,K; I,L,M,V; F,Y,W.

The paragraph beginning at page 8, line 14, and ending at page 8, line 20 has been amended as follows:

In particular, in amino acid sequence of LYC3, there exists a 19 amino acids signature sequence of lysozyme and alpha-lactoalbumin: **CX_3CX_2(L/M/F)X_3(D/E/N)(L/I)X_5C** (SEQ ID NO. 10) [Note: In the sequence, X represents any amino acid, digits such as "2" denote the number of amino acid, "(L/M/H)" represents any of these three amino acids]. Lysozyme and alpha-lactoalbumin are two proteins related closely in evolution (Eur. J. Biochem. 182: 111-118). In the protein of the present invention, the sequence matching the signature is: CRMYCSDLLNPNLKDTVIC (residues 93-111 in SEQ ID NO: 4). It indicates that the LYC3 of the present invention belongs to lysozyme family, and has the relative functions of the lysozyme family.

A NOVEL HUMAN LYSOZYME GENE, ITS ENCODED POLYPEPTIDE AND THE METHOD FOR PREPARING THEM

Field of invention

The invention relates to a new polynucleotide, the polypeptide encoded by said polynucleotide, the uses of said polynucleotide and polypeptide, and the methods for preparing same. In particular, the polypeptide of the invention is identified as a new member of the lysozyme family.

Prior art

Lysozyme exists ubiquitously in all parts of organisms, including various tissues, organs, and sera; it is especially abundant in egg white. Lysozyme is mainly secreted by the epithelial cell of certain glands and some kinds of leukocyte.

Lysozyme was first reported by Fleming, et al. in 1922. Afterward, lysozyme has been widely studied. A lot of papers concerning its crystal structure, protein catalytic domains, catalytic dynamics, immunology, molecular evolutionary, and so on, have been published. Lysozyme is one of the proteins that are studied most extensively and intensively. However, the study on lysozyme gene is not yet sufficient. Nowadays, only a few lysozyme genes from different species, such as E.coli T4, salmonella P22 phage, bacillus Φ phage and chicken, etc., have been cloned. (1983 J. Mol. Biol. 165. 229-248; 1985 Virology 143, 280-289; 1987 Proc. Natl. Acad. Sci. USA, 77, 5759-5763). The cloning about human lysozyme gene was also reported (1988, Gene 66,223-234).

The main function of lysozyme is to hydrolyze the beta(1-4) glycosidic bond between N-acetylmuramic acid (NAM) and N-acetylgluconic acid (NAG) of the bacterial cell wall. In the organism, lysozyme can act as a nonspecific immune molecule against bacterial infections, and as a digestive enzyme in enteron and some mollusks which live on bacteria. Further, lysozyme has the function of inhibiting tumor growth. Therefore, lysozyme has important applications in both industry and medicine.

Summary of Invention

One purpose of the invention is to provide a new polynucleotide which encodes a new member of lysozyme gene family. The new human lysozyme is named LYC3.

Another purpose of the invention is to provide a new member of lysozyme protein family, which is named LYC3.

Still another purpose of the invention is to provide a new method for preparing said new human lysozyme by recombinant techniques.

The invention also relates to the uses of said human lysozyme and its coding sequence.

In one aspect, the invention provides an isolated DNA molecule, which comprises a nucleotide sequence encoding a polypeptide having human LYC3 protein activity, wherein said nucleotide sequence shares at least 70% homology to the nucleotide sequence of nucleotides 81-521 in SEQ ID NO: 3, or said nucleotide sequence can hybridize to the nucleotide sequence of nucleotides 81-521 in SEQ ID NO: 3

under moderate stringency. Preferably, said nucleotide sequence encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 4 or of 20-148 in SEQ ID NO: 4. More preferably, the sequence comprises the nucleotide sequence of nucleotides 81-521 in SEQ ID NO: 3.

Further, the invention provides an isolated LYC3 polypeptide, which comprises a polypeptide having the amino acid sequence of SEQ ID NO: 4 or of 20-148 in SEQ ID NO: 4, its active fragments, and its active derivatives. Preferably, the polypeptide is a polypeptide having the amino acid sequence of SEQ ID NO: 4 .

The invention also provides a vector comprising said isolated DNA.

The invention further provides a host cell transformed with said vector.

In another aspect, the invention provides a method for producing a polypeptide with the activity of LYC3 protein, which comprises:

(a) forming an expression vector of LYC3 protein comprising the nucleotide sequence encoding the polypeptide having the activity of LYC3 protein, wherein said nucleotide sequence is operably linked with an expression regulatory sequences, and said nucleotide sequence shares at least 70% homology to the nucleotide sequence of positions 81-521 in SEQ ID NO: 3;

(b) introducing the vector of step (a) into a host cell, thereby forming a recombinant cell of LYC3 protein;

(c) culturing the recombinant cell of step (b) under the conditions suitable for the expression of LYC3 polypeptides;

(d) isolating the polypeptides having the activity of LYC3 protein.

In one embodiment of the present invention, the isolated polynucleotide has a full length of 544 nucleotides, whose detailed sequence is shown in SEQ ID NO: 3. The open reading frame (ORF) locates at nucleotides 81-521.

In the present invention, the term "isolated" or "purified" or "substantially pure" DNA refers to a DNA or fragment which has been isolated from the sequences which frank it in a naturally occurring state. The term also applied to DNA or DNA fragment which has been isolated from other components naturally accompanying the nucleic acid and from proteins naturally accompanying it in the cell.

In the present invention, the term "LYC3 protein encoding sequence" or " LYC3 polypeptide encoding sequence " refers to a nucleotide sequence encoding a polypeptide having the activity of LYC3 protein, such as the nucleotide sequence of positions 81-521 in SEQ ID NO: 3 or its degenerate sequence. The degenerate sequences refer to the sequences formed by replacing one or more codons in the ORF of 81-521 in SEQ ID NO: 3 with degenerate codes which encode the same amino acid. Because of the degeneracy of codon, the sequence having a homology as low as about 70% to the sequence of nucleotides 81-521 in SEQ ID NO: 3 can also encode the sequence shown in SEQ ID NO: 4. The term also refers to the nucleotide sequences that hybridize with the nucleotide sequence of nucleotides 81-521 in SEQ ID NO: 3 under moderate stringency or preferably under high stringency. In addition, the term also refers to the sequences having a homology at least 70%, preferably 80%, more preferably 90% to the nucleotide sequence of nucleotides 81-521 in SEQ ID NO: 3. Moreover, the term includes a nucleotide sequence encoding a

mature protein without signal peptide, such as the nucleotide sequence of position 135-521 in SEQ ID NO: 3.

The term also refers to variants of the sequence in SEQ ID NO: 3, which are capable of coding for a protein having the same function as human LYC3 protein. These variants includes, but are not limited to: deletions, insertions and/or substitutions of several nucleotides (typically 1-90, preferably 1-60, more preferably 1-20, and most preferably 1-10) and additions of several nucleotides (typically less than 60, preferably 30, more preferably 10, most preferably 5) at 5' end and/or 3' end.

In the present invention, "substantially pure" proteins or polypeptides refers to those which occupy at least 20%, preferably at least 50%, more preferably at least 80%, most preferably at least 90% of the total sample material (by wet weight or dry weight). Purity can be measured by any appropriate method, e.g., in the case of polypeptides by column chromatography, PAGE or HPLC analysis. A substantially purified polypeptides is essentially free of naturally associated components.

In the present invention, the term "LYC3 polypeptide "or "LYC3 protein " refers to a polypeptide having the activity of LYC3 protein comprising the amino acid sequence of SEQ ID NO: 4 or of positions 20-148 of SEQ ID NO: 4. The term also comprises the variants of said amino acid sequence which have the same function of human lysozyme. These variants include, but are not limited to, deletions, insertions and/or substitutions of several amino acids (typically 1-50, preferably 1-30, more preferably 1-20, most preferably 1-10), and addition of one or more amino acids (typically less than 20, preferably less than 10, more preferably less than 5) at C-terminal and/or N-terminal. For example, the protein function are usually unchanged when an amino residue is substituted by a similar or analogous one. Further, the addition of one or several amino acids at C-terminal and/or N-terminal will not change the function of protein. The term also includes the active fragments and derivatives of LYC3 protein.

The variants of polypeptide include homologous sequences, allelic variants, natural mutants, induced mutants, proteins encoded by DNA which hybridizes to LYC3 DNA under high or low stringency conditions as well as the polypeptides or proteins retrieved by antisera raised against LYC3 polypeptide. The present invention also provides other polypeptides, e.g., fusion proteins, which include the LYC3 polypeptide or fragments thereof. In addition to substantially full-length polypeptide, the soluble fragments of LYC3 polypeptide are also provided. Generally, these fragments comprise at least 10, typically at least 30, preferably at least 50, more preferably at least 80, most preferably at least 100 consecutive amino acids of human LYC3 polypeptide.

The present invention also provides the analogues of LYC3 protein or polypeptide. Analogues can differ from naturally occurring LYC3 polypeptide by amino acid sequence differences or by modifications which do not affect the sequence, or by both. These polypeptides include genetic variants, both natural and induced. Induced variants can be made by various techniques, e.g., by random mutagenesis using irradiation or exposure to mutagens, or by site-directed mutagenesis or other known molecular biologic techniques. Also included are analogues which include residues other than those naturally occurring L-amino acids (e.g., D-amino acids) or non-naturally occurring or synthetic amino acids (e.g., beta- or gamma-amino acids). It is understood that the polypeptides of the invention are not limited to the representative polypeptides listed hereinabove.

Modifications (which do not normally alter primary sequence) include in vivo, or in vitro chemical derivation of polypeptides, e.g., acelylation, or carboxylation. Also included are modifications of glycosylation, e.g., those made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing or in the further processing steps, e.g., by exposing the polypeptide to enzymes which affect glycosylation (e.g., mammalian glycosylating or deglycosylating enzymes). Also included are sequences which have phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, phosphothronine, as well as sequences which have been modified to improve their resistance to proteolytic degradation or to optimize solubility properties.

The invention also includes antisense sequence of the sequence encoding LYC3 polypeptide. Said antisense sequence can be used to inhibit expression of LYC3 in cells.

The invention also include probes, typically having 8-100, preferably 15-50 consecutive nucleotides. These probes can be used to detect the presence of nucleic acid molecules coding for LYC3 in samples.

The present invention also includes methods for detecting LYC3 nucleotide sequences, which comprises hybridizing said probes to samples, and detecting the binding of the probes. Preferably, the samples are products of PCR amplification. The primers in PCR amplification correspond to coding sequence of LYC3 polypeptide and are located at both ends or in the middle of the coding sequence. In general, the length of the primers is 20 to 50 nucleotides.

A variety of vectors known in the art, such as those commercially available, are useful in the invention.

In the invention, the term "host cells" includes prokaryotic and eukaryotic cells. The common prokaryotic host cells include Escherichi coli, Bacillus subtilis, and so on. The common eukaryotic host cells include yeast cells, insect cells, and mammalian cells. Preferably, the host cells are eukaryotic cells, e.g., CHO cells, COS cells, and the like.

In another aspect, the invention also includes antibodies, preferably monoclonal antibodies, which are specific for polypeptides encoded by LYC3 DNA or fragments thereof. By "specificity" is meant an antibody which binds to the LYC3 gene products or a fragments thereof. Preferably, the antibody binds to the LYC3 gene products or a fragments thereof and does not substantially recognize and bind to other antigenically unrelated molecules. Antibodies which bind to LYC3 and block LYC3 protein and those which do not affect the LYC3 function are included in the invention. The invention also includes antibodies which bind to the LYC3 gene product in its unmodified as well as modified form.

The present invention includes not only intact monoclonal or polyclonal antibodies, but also immunologically-active antibody fragments, e.g., a Fab' or $(Fab)_2$ fragment, an antibody light chain, an antibody heavy chain, a genetically engineered single chain Fv molecule (Lander, et al.,US Pat No. 4,946,778), or a chimeric antibody, e.g., an antibody which contains the binding specificity of a murine antibody, but the remaining portion of which is of human origin.

The antibodies in the present invention can be prepared by various techniques known to those skilled in the art. For example, purified LYC3 gene products, or its antigenic fragments can be administrated to animals to induce the production of polyclonal antibodies. Similarly, cells expressing LYC3 or its antigenic fragments can be used to immunize animals to produce antibodies. Antibodies of the invention can be monoclonal antibodies which can be prepared by using hybridoma technique (See Kohler, et al., Nature,

256; 495,1975; Kohler, et al., Eur. J. Immunol. 6: 511,1976; Kohler, et al., Eur. J. Immunol. 6: 292, 1976; Hammerling, et al., In Monoclonal Antibodies and T Cell Hybridomas, Elsevier, N.Y., 1981). Antibodies of the invention comprise those which block LYC3 function and those which do not affect LYC3 function. Antibodies in the invention can be produced by routine immunology techniques and using fragments or functional regions of LYC3 gene product. These fragments and functional regions can be prepared by recombinant methods or synthesized by a polypeptide synthesizer. Antibodies binding to unmodified LYC3 gene product can be produced by immunizing animals with gene products produced by prokaryotic cells (e.g., E. coli); antibodies binding to post-translationally modified forms thereof can be acquired by immunizing animals with gene products produced by eukaryotic cells (e.g., yeast or insect cells).

The full length human LYC3 nucleotide sequence or its fragment of the invention can be prepared by PCR amplification, recombinant method and synthetic method. For PCR amplification, one can obtain said sequences by designing primers based on the nucleotide sequence disclosed in the invention, especially the sequence of ORF, and using cDNA library commercially available or prepared by routine techniques known in the art as a template. When the sequence is long, it is usually necessary to perform two or more PCR amplifications and link the amplified fragments together in the correct order.

Once the sequence is obtained, a great amount of the sequences can be produced by recombinant methods. Usually, said sequence is cloned in a vector which is transformed into a host cell. Then the sequence is isolated from the amplified host cells using conventional techniques.

In addition to recombinant techniques, the protein fragments of the invention may also be prepared by direct chemical synthesis using solid phase synthesis techniques (Stewart et al., (1969) Solid-Phase Peptide Synthesis, WH Freeman Co., San Francisco; Merrifield J. (1963), J. Am. Chem. Assoc. 85: 2149-2154). In vitro protein synthesis can be performed manually or automatically, e.g., using a Model 431 Peptide Synthesizer (Applied Biosystems, Foster City, CA). The fragments of protein of the invention can be synthesized separately and linked together using chemical methods so as to produce full-length molecule.

The sequences encoding the protein of the present invention are also valuable for gene mapping. For example, the accurate chromosome mapping can be performed by hybridizing cDNA clones to a chromosome in metaphase. This technique can use cDNA as short as about 500bp, or as long as about 2000bp, or more. For details, see Verma et al., Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found in, e.g., Mendelian Inheritance in Man (available on-line through Johns Hopkins University Welch Medical Library). The relationships between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis.

Then, the differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individual, then the mutation is likely to be the causative agent of the disease.

The substances which act with the LYC3, e.g., receptors, inhibitors and antagonists, can be screened

out by various conventional techniques and using the protein of the invention.

The protein, antibody, inhibitor, antagonist or receptor of the invention provide different effects when administrated in therapy. Usually, these substances are formulated with a non-toxic, inert and pharmaceutically acceptable aqueous carrier. The pH typically ranges from 5 to 8, preferably about 6-8, although pH may alter according to the property of the formulated substances and the diseases to be treated. The formulated pharmaceutical composition is administrated in conventional routine including, but not be limited to, intramuscular, intraperitoneal, subcutaneous, intracutaneous, or topical administration.

As an example, the human LYC3 protein of the invention may be administrated together with the suitable and pharmaceutically acceptable carrier. The examples of carriers include, but are not limited to, saline, buffer solution, glucose, water, glycerin, or the combination thereof. The pharmaceutical formulation should be suitable for the delivery method. The human LYC3 protein of the invention may be in the form of injections which are made by conventional methods and using physiological saline or other aqueous solution containing glucose or auxiliary substances. The pharmaceutical compositions in the form of tablet or capsule may be prepared by routine methods. The pharmaceutical compositions, e.g., injections, solutions, tablets, and capsules, should be manufactured under sterile conditions. The active ingredient is administrated in therapeutically effective amount, e.g., from about 1ug to 5mg per kg body weight per day. Moreover, the polypeptide of the invention can be administrated together with other therapeutic agent.

When the human LYC3 polypeptides of the invention are used as a pharmaceutical, the therapeutically effective amount of the polypeptides are administrated to mammals. Typically, the therapeutically effective amount is at least about 10 ug/kg body weight and less than about 8 mg/kg body weight in most cases, and preferably about 10ug-1mg/kg body weight. Of course, the precise amount will depend upon the factors, such as delivery methods, the subject health, and the like, and is within the judgment of the skilled clinician.

In one embodiment, the polynucleotide of the invention is 544 bp in full length whose detailed sequence is shown in SEQ ID NO: 3 with the ORF located at positions 81-521. Said polynucleotide was obtained as follows: human brain λ gt 11 cDNA library (Clontech) was used as a template and PCR was carried out with the synthetic forward primer A1 : 5'-AGAGTGGTGGTGGCTCCACTCTG-3' and reverse primer B :5'-TGCTGTGCATGGTTCCGTCCATC-3'. A target fragment of 544bp was obtained. The sequencing of the PCR product gave the full length cDNA sequence shown in SEQ ID NO: 3.

Homology comparison showed that the nucleotide sequence and the coded protein sequence of the invention shared remarkable homology to other lysozymes from different origins. Therefore, it indicates it is a new member of lysozyme family and has some important functions of the family.

Lysozyme can lyse cells by hydrolyze the beta(1-4) glycosidic bond between N-acetylmuramic acid (NAM) and N-acetylgluconic acid (NAG) of the bacterial cell wall. In the organisms, lysozyme can act as a nonspecific immune molecule against bacterial infections, and as a digestive enzyme in enteron and some mollusks which live on bacteria. Further, lysozyme has the function of inhibiting tumor growth. In 1955, Caselli and Shumacher (Boll Ocul 34:513-533, 1955) reported on the lysozyme-mediated 70% inhibition of neoplastic transformation in cornea of chicken infected by Rous sarcoma virus. Many other experiments indicated that lysozyme participates in the process of tumor diffusion and interacts with phospho- and

glucolipid molecule of tumor cells. The inhibition effect on human tumor of lysozyme was reported and patented (1980 Jpn Kokai, Tokkyo Koho 33,409; 1980 Jpn Kokai Tokkyo Koho 33,408). As to the mechanism of lysozyme inhibition on tumor, there are two possibilities: (1) lysozyme directly activates the organism's immunity functions; (2) lysozyme indirectly enhances the organism's immune ability (1989 Anticancer Research 9, 583-592).

Description of Drawings

Fig. 1 shows an alignment comparison of amino acid sequences of human LYC3 and other lysozymes. Fig. 1A shows a homology comparison of amino acid sequences of human LYC3 and lysozyme C of *Trachypithecus francoisi* (gi|1790947). Fig. 1B shows a homology comparison of amino acid sequences of human LYC3 and lysozyme C of ring-necked pheasant (sp|p00702). The identical amino acids are indicated by ":" between the sequences, and the similar amino acids indicated by ".". The similar amino acids are as follows: A,S,T; D,E; N,Q; R,K; I,L,M,V; F,Y,W.

Fig. 2 shows the bacteriolysis effect of LYC3 of the invention.

The invention is further illustrated by the following examples. It is appreciated that these examples are only intended to illustrate the invention, but not to limit the scope of the invention. For the experimental methods in the following examples, they are performed under routine conditions, e.g., those described by Sambrook. et al., in Molecule Clone: A Laboratory Manual, New York: Cold Spring Harbor Laboratory Press, 1989, or as instructed by the manufacturers, unless otherwise specified.

Examples

Example 1

The cloning and sequencing of LYC3 cDNA sequence

1. Amplification with primers

The template was human brain λ gt 11 cDNA library (commercially available from Clontech). PCR with forward primer A1 : 5'-AGAGTGGTGGTGGCTCCACTCTG-3' (SEQ ID NO: 1) and reverse primer B :5'-TGCTGTGCATGGTTCCGTCCATC-3' (SEQ ID NO: 2) was carried out. The PCR condition for A1/B was 4 mins at 93°C; followed by 35 cycles with 1 min at 93°C, 1 min at 69°C, and 1 min at 72°C; and, finally 5 mins at 72°C. The PCR fragments were detected by electrophoresis. The target fragment was 544bp.

2. Sequencing PCR products

The obtained PCR products were linked with pGEM-T® vector (Promega) and transformed into E. coli JM103. The plasmids were extracted using QIAprep Plasmid Kit (QIAGEN). The oriented serial deletion of the inserted fragments was carried out with Double-Stranded Nested Deletion Kit (Pharmacia), and the deletants were quickly identified by PCR and arranged in order. The deletants successively cut-off were sequenced with SequiTherm EXCEL™ DNA Sequencing Kit (Epicentre Technologies). A full length cDNA sequence of 544bp was obtained by overlapping the sequences with computer software. The detailed

sequence is shown in SEQ ID NO: 3 with an open reading frame (ORF) located at nucleotides 81-521.

According to the resultant full-length cDNA sequence, the amino acid sequence of LYC3 was deduced, having 146 amino acid residues totally. See SEQ ID NO: 4 for its amino acid sequence in details.

Example 2

Homologous comparison

The full length cDNA sequence of LYC3 and the coded protein were used for homologous screening Non-redundant GenBank + EMBL + DDBJ + PDB and GenBank CDS translations + PDB + SwissProt + Spupdate + PIR databases by BLAST algorithm. The result showed that they shared high homology to other members of the lysozyme family. The amino acid sequence of LYC3 shares 51.4% identity and 64.4% similarity with *Trachypithecus francoisi* lysozyme C (gi|1790947) (Fig. 1A), and 46.6% identity and 59.2% similarity with lysozyme C of ring-necked pheasant (sp|p00702)(Fig. 1B), when analyzed by PCGENE software.

In particular, in amino acid sequence of LYC3, there exists a 19 amino acids signature sequence of lysozyme and alpha-lactoalbumin: **CX_3CX_2(L/M/F)X_3(D/E/N)(L/I)X_5C** [Note: In the sequence, X represents any amino acid, digits such as "2" denote the number of amino acid, "(L/M/H)" represents any of these three amino acids]. Lysozyme and alpha-lactoalbumin are two proteins related closely in evolution (Eur. J. Biochem. 182: 111-118). In the protein of the present invention, the sequence matching the signature is: CRMYCSDLLNPNLKDTVIC (residues 93-111 in SEQ ID NO: 4). It indicates that the LYC3 of the present invention belongs to lysozyme family, and has the relative functions of the lysozyme family.

The human LYC3 protein has a signal peptide of 18 amino acids (residues 1-18 of SEQ ID NO: 4). After cleavage of the signal peptide, the mature human LYC3 protein has the amino acid sequence of residues 19-146 of SEQ ID NO: 4.

Lysozyme can lyse cells by hydrolyze the beta(1-4) glycosidic bond between N-acetylmuramic acid (NAM) and N-acetylgluconic acid (NAG) of the bacterial cell wall. In the organisms, lysozyme can act as a nonspecific immune molecule against bacterial infections, and as a digestive enzyme in enteron and some mollusks which live on bacteria.

Lysozyme has important applications in both industry and medicine.

First, in industry (mainly in food industry), lysozyme can be used as a preservative or additive for food. In this respect, the Japanese have developed many use of lysozyme and owe many patents. For example, they use lysozyme as a preservative for fresh fruit, vegetable, soybean milk, marine foods and meat. Lysozyme can also be used as an additive for infant's foods to simulate human milk (1988, Crit Rev Food Sci Nutr 26(4):359-395).

In respect of pharmaceutical use, lysozyme can be used to cure viral and bacterial infections. For example, EDTA-tris-lysozyme solutions are effective on the pseudomonas cystitis induced by E.coli infection. Lysozyme concentration in human and animal serum is an indicator of infection. Zajaczkowska-Bialowas and Murai studied the relationship between lysozyme activity in saliva and diseases of oral cavity. The result showed that lysozyme had obvious alleviation effect on the symptom of chronic periodontitis. Besides, they found the synergistic effects of lysozyme and some antibiotics. When lysozyme was used

alone, even in a large amount, the bacteriolysis effect on *S. aureus* was little. But with the presence of amoxicillin, the lysis effect was enhanced and in proportion to the amount of lysozyme (1988 Crit Rev Food Sci Nutr 26(4):359-395).

Further, lysozyme has the function of inhibiting tumor growth. In 1955, Caselli and Shumacher (1955, Boll Ocul 34:513-533) reported on the lysozyme-mediated 70% inhibition of neoplastic transformation in cornea of chicken infected by Rous sarcoma virus. Many other experiments indicated that lysozyme had some relationship to the inhibition of tumor diffusion (1988 Clin. Expl. Metastasis 6:245-253; 1998 Folia Onclo 10, Suppl A: 219-224; 1988 Eur. J. Cancer Clin. Onco. 124:1737-1743). It is also found that lysozyme interacts with phospho- and glucolipid molecule of tumor cells. The lysozyme's inhibition effect on human tumor was reported. Laterza successfully cured a case of small intestine reticulation sarcoma with diffusion after operation and radiotherapy ("Atti del II Simposium Internazionale sul Lisozima ", Milano. 7-8-9 1961. Vol I, sez V, pp 49-50). Battaglia et al. found that, though lysozyme could not reduce the volume of tumor, it had distinct effects of pain-killing and helping recovery in curing carcinomas of stomach, prostate, uterus and mammary gland ("Atti del II Simposium Internaionale sul Lisozima di Fleming", Milano. 3-4-5 1964. Vol I, sez IV, pp 69-76). In Japan, the application of lysozyme in curing cancer was patented (1980 Jpn Kokai Tokkyo Koho 33, 409; 1980 Jpn Kokai Tokkyo Koho 33,408). Besides, A. Vacca et al. in 1985 reported an attempt of curing multiple myeloma by chemoimmunology with oral lysozyme as an immunomodulating agent. Their experiments indicated that 50% of the patients treated with a large amount of lysozyme had improved immune ability as compared with the controls (Chemiother IV n.2:147-155,1985). As to the mechanism of lysozyme inhibition on tumor, there are two possibilities: (1) lysozyme directly activates the organism's immunity functions; (2) lysozyme indirectly enhances the organism's immune ability (1989 Anticancer Research 9, 583-592).

Example 3

Expression of LYC3 in E. coli

The cDNA sequence encoding LYC3 was amplified with oligonucleotide PCR primers corresponding to 5'- and 3'-end of said DNA sequence, using human brain λ gt 11 cDNA library (Clontech) as a template. The resultant product was used as an insertion fragment.

The sequence of 5'-end oligonucleotide primer was:

5'-TCTCGGATCCATGTTGTTGGCCCTGGTCT-3' (SEQ ID NO: 5).

This primer contained a cleavage site of restriction endonuclease BamH I, followed by 19 nucleotides of LYC3 coding sequence starting from the start codon.

The sequence of 3'-end primer was:

5'-CCTTGTCGACCTAGAAGTCACAGCCATCC-3'(SEQ ID NO: 6).

This primer contains a cleavage site of restriction endonuclease SalI, a translation terminator and partial LYC3 coding sequence.

These cleavage sites of restriction endonuclease in primers corresponded to the cleavage sites in bacterial expression vector pQE-9 (Qiagen Inc., Chatsworth, CA). Vector pQE-9 encodes an antibiotic resistance (Amp^r), a bacterial replication origin (ori), an IPTG-adjustable promotor/operon (P/O), a

ribosome-binding site (RBS), a six-hisitine tag (6-His) and cloning sites of restriction endonuclease.

Vector pQE-9 and insertion fragments were digested by BamHI and SalI, and then linked together, ensuring that the open reading frame started from the bacterial RBS. Then, the linkage mixture was used to transform E.coli M15/rep4 (Qiagen) containing multi-copy of plasmid pREP4 which expressed repressor of lacI and was resistant to kanamycin (Kan^r). Transformants were screened out in LB medium containing Amp and Kan. The positive clones of transformant were cultured overnight in LB liquid medium supplemented with Amp (100ug/ml) and Kan (25ug/ml). The plasmids were extracted. The size and direction of the inserted fragments were verified by HindIII digestion. The sequencing confirmed that LYC3 cDNA fragment was correctly inserted into the vector.

The overnight culture was 1:100-1:250 diluted, inoculated into large volume medium, and cultured until the 600nm optical density (OD_{600}) reached 0.4-0.6. IPTG (isopropylthio-beta-D-galactoside) was added to final concentration of 1mM. By deactivating repressor of LacI, IPTG induced and promoted P/O, thereby increasing the expression of gene. The cells were cultured for another 3-4 hours, and then centrifuged (6000Xg, 20 mins). The cultures were sonicated, and cell lysate was collected and diluted with 6M guanidine hydrochloride. After clarification, the dissolved LYC3 in solution were purified by nickel-chelated column chromatography under the conditions suitable for the tight binding of 6-His tagged protein and column. LYC3 was eluted with 6M-guanidine hydrochloride (pH 5.0). The denaturalized proteins in guanidine hydrochloride were precipitated by several methods. First, guanidine hydrochloride was separated by dialysis. Alternatively, the purified protein, which was isolated from nickel-chelated column, bound to the second column with decreased linear gradient of guanidine hydrochloride. The proteins were denatured when binding to the column, and then eluted with guanidine hydrochloride (pH 5.0). Finally, the soluble proteins were dialyzed with PBS, then preserved in glycerol stock solution with the final glycerol concentration of 10% (w/v).

The molecular weight of the expressed protein was 16 kDa, as identified by 12% SDS-PAGE.

Moreover, the sequencing results of the 10 amino acids at the N- and C-terminal of the expressed protein indicated that they were identical to those in SEQ ID NO: 4.

Example 4

Expression of LYC3 in eukaryotic cells (CHO cell line)

In this example, the cDNA sequence encoding LYC3 was amplified with oligonucleotide PCR primers corresponding to 5'- and 3'-end of said DNA sequence, using human brain λ gt 11 cDNA library (Clontech) as a template. The resultant product was used as an insertion fragment.

The sequence of 5'-end oligonucleotide primer was:

5'-TCTCAAGCTTATGTTGTTGGCCCTGGTCT-3'(SEQ ID NO: 7),

This primer contained a cleavage site of restriction endonuclease HindIII, followed by 20 nucleotides of LYC3 coding sequence starting from the start codon.

The sequence of 3'-end primer was:

5'-CCTTGGATCCCTAGAAGTCACAGCCATCC-3' (SEQ ID NO: 8)

The primer contained a cleavage site of restriction endonuclease BamHI, a translation stop codon, and

partial LYC3 coding sequence.

These cleavage sites of restriction endonuclease in primers corresponded to the cleavage sites in expression vector pcDNA3 for CHO cell. This vector encoded two kinds of antibiotic resistance (Amp^r and Neo^r), a phage replication origin (fl ori), a virus replication origin (SV40 ori), a T7 promoter, a virus promoter (P-CMV), a Sp6 promoter, a polyadenylation signal of SV40 and the corresponding polyA sequence thereof, a polyadenylation signal of BGH and the poly A sequence thereof.

The vector pcDNA3 and insertion fragment were digested with HindIII and BamHI, and linked together. Subsequently, E.coli strand DH5 α was transformed with linkage mixture. Transformants were screened out in LB medium containing Amp. The clones containing the needed constructs were cultured overnight in LB liquid medium supplemented with Amp (100 ug/ml). Plasmids were extracted. The sequencing indicated that LYC3 cDNA fragment was correctly inserted into the vector.

Plasmids were transfected into CHO cells by lipofection with Lipofectin Kit (GIBco Life). After transfecting the cells for 48 hours and screening the cells with G418 for 2-3 weeks, the cells and cell supernatant were collected and the enzyme activity of the expressed protein was measured. G418 was removed and the transformants were subcultured continuously. The mixed clonal cells were limiting diluted and the subclones with higher protein activity were selected. The positive subclones were mass cultured by routine methods. 48 hours later, the cells and supernatant were collected. The cells were ultrasonicated. Using 50mM Tris-HCl (pH7.6) solution containing 0.05% Triton as an equilibrium solution and eluent, the active peek of the protein was collected with a pre-balanced Superdex G-75 column. Then, using 50mM Tris-HCl (pH8.0) solution containing 0-1 M NaCl as an eluent, the protein was gradiently washed on a DEAE-Sepharose column balanced with 50mM Tris-HCl (pH8.0) solution. The active peek of the protein was collected. The solution of the expressed protein was dialyzed with PBS (pH7.4), and finally lyophilized and preserved.

The molecular weight of the expressed protein was 15 kDa as identified by 12% SDS-PAGE.

Meanwhile, a 5'-end oligonucleotide primer was designed to remove signal peptide: 5'-TCTCAAGCTT AAGCTCTACG GTCGTTG-3' (SEQ ID NO: 9). The above procedure of Example 4 was repeated except that SEQ ID NOs: 9 and 8 were used as primers. An expressed protein with the molecular weight of 15 kDa was obtained, which was LYC3 without the signal peptide.

Moreover, the sequencing results of the 10 amino acids at the N- and C-terminal of the expressed protein indicated that they were identical to those in SEQ ID NO: 4.

Example 5

Antibody preparation

Antibodies were produced by immunizing animals with the recombinant proteins obtained in the above examples. The method was as follows: the recombinant proteins were isolated by chromatography, and stored for use. Alternatively, the protein was isolated by SDS-PAGE electrophoresis, and obtained by cutting eletrophoretic bands from gel. The protein was emulsified with Freund's complete adjuvant of the same volume. The emulsified protein was injected intraperitoneally into mice at a dosage of 50-100ug/0.2ml. 14 days later, the same antigen was emulsified with Freund's incomplete adjuvant and

injected intraperitoneally into mice at a dosage of 50-100ug/0.2ml for booster immunization. Booster immunization was carried out every 14 days, for at least three times. The specific activity of the obtained antiserum was evaluated by its ability of precipitating the translation product of LYC3 gene in vitro.

Example 6

The bacteriolysis effect of LYC3

In the similar manner described in Example 3, the LYC3 gene was amplified using primer with EcoRI and primer with XhoI sites. Then, the LYC3 amplification product was digested by EcoRI and XhoI, and cloned into vector pPIC9K (Invitrogen). The vector was digested by SalI and transformed into *Pichia pastoris* by electroporation. The yeast in which LYC3 gene was incorporated was screened out on a His(-) medium.

The supernatant of the culture of *Pichia pastoris* incorporated with LYC3 was used as a sample, and diluted in serial in 1:1, 1:5, 1:10, 1:20; 1:30, and 1:50. The supernatant of the culture of *Pichia pastoris* not incorporated with LYC3 was used as a control. 100 ul of sample or control was preheated at 37 °C for 2 mins. The substrate (*Micrococcus lysodeikticus* marked with Red Dye K-2BP and suspended in 0.5M phosphate buffer, pH 6.5) preheated at 37 °C was added and reacted at 37 °C for 20 mins. 400Ul of emulsifier was added to stop the reaction. The reaction mixture was centrifugated 4000 rpm 5 mins and the supernatant was taken and observed by naked eye. The samples were redder than the control. The reaction liquid was read on a Model 721 spectrophotometer at 540 wave length and a blank tube was used as a control. The absorbance of the sample were larger than that of the control. See Fig. 2A. On the top were the lysozyme products commercially available from Sigma. From left to right were 10^{-2}, $5x10^{-3}$,$2x10^{-3}$,$5x10^{-4}$, and 10^{-4} mg lysozyme and the control. Below were the culture supernatants of LYC3 protein of the invention, and from left to right were 1:1, 1:5, 1:10, 1:20; 1:30, and 1:50 diluted LYC3 supernatant, and the control. The deeper red color indicated that more bacteria were lysed. LYC3 had bacteriolysis effect as shown in Fig. 2A.

All the documents cited herein are incorporated into the invention as reference, as if each of them is individually incorporated. Further, it is appreciated that, in the above teaching of the invention, the skilled in the art can make certain changes or modifications to the invention, and these equivalents are still within the scope of the invention defined by the appended claims of the present application.

SEQUENCE LISTING

(2) INFORMATION FOR SEQ ID NO: 1:
(i)SEQUENCE CHARACTERISTICS:
(A)LENGTH: 23bp
(B)TYPE: nucleic acid
(C)STRANDEDNESS: single
(D)TOPOLOGY: linear
(ii)MOLECULAR TYPE: oligonucleotide
(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 1:

AGAGTGGTGG TGGCTCCACT CTG 23

(2) INFORMATION FOR SEQ ID NO: 2:
(i)SEQUENCE CHARACTERISTICS:
(A)LENGTH: 23bp
(B)TYPE: nucleic acid
(C)STRANDEDNESS: single
(D)TOPOLOGY: linear
(ii)MOLECULAR TYPE: oligonucleotide
(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 2:

TGCTGTGCAT GGTTCCGTCC ATC 23

(2) INFORMATION FOR SEQ ID NO: 3:
(i)SEQUENCE CHARACTERISTICS:
(A)LENGTH: 544bp
(B)TYPE: nucleic acid
(C)STRANDEDNESS: double
(D)TOPOLOGY: linear
(ii)MOLECULAR TYPE: cDNA
(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
  1 AGAGTGGTGG TGGCTCCACT CTGCCGCCGC ATAGAAGCCA GGAGCAGGGC TCTCAGAAGG
 61 CGGTGGTGCC AGCTGGGATC ATGTTGTTGG CCCTGGTCTG TCTGCTCAGC TGCCTGCTAC
121 CCTCCAGTGA GGCCAAGCTC TACGGTCGTT GTGAACTGGC CAGAGTGCTA CATGACTTCG
181 GGCTGGACGG ATACCGGGGA TACAGCCTGG CTGACTGGGT CTGCCTTGCT TATTTCACAA
241 GCGGTTTCAA CGCAGCTGCT TTGGACTACG AGGCTGATGG GAGCACCAAC AACGGGATCT
301 TCCAGATCAA CAGCCGGAGG TGGTGCAGCA ACCTCACCCC GAACGTCCCC AACGTGTGCC
361 GGATGTACTG CTCAGATTTG TTGAATCCTA ATCTCAAGGA TACCGTTATC TGTGCCATGA
421 AGATAACCCA AGAGCCTCAG GGTCTGGGTT ACTGGGAGGC CTGGAGGCAT CACTGCCAGG
481 GAAAAGACCT CACTGAATGG GTGGATGGCT GTGACTTCTA GGATGGACGG AACCATGCAC
541 AGCA
```

(2) INFORMATION FOR SEQ ID NO: 4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 146 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: lineal
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
 1 Met Leu Leu Ala Leu Val Cys Leu Leu Ser Cys Leu Leu Pro Ser
16 Ser Glu Ala Lys Leu Tyr Gly Arg Cys Glu Leu Ala Arg Val Leu
31 His Asp Phe Gly Leu Asp Gly Tyr Arg Gly Tyr Ser Leu Ala Asp
46 Trp Val Cys Leu Ala Tyr Phe Thr Ser Gly Phe Asn Ala Ala Ala
61 Leu Asp Tyr Glu Ala Asp Gly Ser Thr Asn Asn Gly Ile Phe Gln
76 Ile Asn Ser Arg Arg Trp Cys Ser Asn Leu Thr Pro Asn Val Pro
91 Asn Val Cys Arg Met Tyr Cys Ser Asp Leu Leu Asn Pro Asn Leu
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106 Lys Asp Thr Val Ile Cys Ala Met Lys Ile Thr Gln Glu Pro Gln
121 Gly Leu Gly Tyr Trp Glu Ala Trp Arg His His Cys Gln Gly Lys
136 Asp Leu Thr Glu Trp Val Asp Gly Cys Asp Phe

(2) INFORMATION FOR SEQ ID NO: 5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29bp
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: oligonucleotide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:
TCTCGGATCC ATGTTGTTGG CCCTGGTCT 29

(2) INFORMATION FOR SEQ ID NO: 6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29bp
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: oligonucleotide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:
CCTTGTCGAC CTAGAAGTCA CAGCCATCC 29

(2) INFORMATION FOR SEQ ID NO: 7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29bp
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: oligonucleotide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:
TCTCAAGCTT ATGTTGTTGG CCCTGGTCT 29

(2) INFORMATION FOR SEQ ID NO: 8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29bp
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: oligonucleotide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:
CCTTGGATCC CTAGAAGTCA CAGCCATCC 29

(2) INFORMATION FOR SEQ ID NO: 9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27bp
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: oligonucleotide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:
TCTCAAGCTT AAGCTCTACG GTCGTTG 27

CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence encoding a polypeptide having human LYC3 protein activity, wherein said nucleotide sequence shares at least 70% homology to the nucleotide sequence of nucleotides 81-521 in SEQ ID NO: 3, or said nucleotide sequence can hybridize to the nucleotide sequence of nucleotides 81-521 in SEQ ID NO: 3 under moderate stringency.

2. The DNA molecule of Claim 1 wherein said nucleotide sequence encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 4 or of 20-148 in SEQ ID NO: 4.

3. The DNA molecule of Claim 1 wherein said nucleotide sequence comprises the nucleotide sequence of nucleotides 81-521 in SEQ ID NO: 3.

4. An isolated LYC3 polypeptide comprising a polypeptide having the amino acid sequence of SEQ ID NO: 4 or of 20-148 in SEQ ID NO: 4, its active fragments, and its active derivatives.

5. The polypeptide of Claim 4 wherein said polypeptide is a polypeptide having the amino acid sequence of SEQ ID NO: 4 or of 20-148 in SEQ ID NO: 4.

6. A vector containing the DNA sequence of Claim 1.

7. A host cell transformed by the vector of Claim 6.

8. The host cell of claim 7 wherein it comprises E.coli.

9. The host cell of claim 7 wherein it comprises eukaryotic cell.

10. A method for producing a method for producing a polypeptide having the activity of LYC3 protein, which comprises the steps of :

(a) forming an expression vector of LYC3 protein comprising the nucleotide sequence encoding the polypeptide having the activity of LYC3 protein, wherein said nucleotide sequence is operably linked with an expression regulatory sequences, and said nucleotide sequence shares at least 70% homology to the nucleotide sequence of positions 81-521 in SEQ ID NO: 3;

(b) introducing the vector of step (a) into a host cell, thereby forming a recombinant cell of LYC3 protein;

(c) culturing the recombinant cell of step (b) under the conditions suitable for expression of LYC3 polypeptides;

(d) isolating the polypeptides having the activity of LYC3 protein.

11. The method of Claim 10 wherein said nucleotide sequence comprises nucleotides 81-521 of SEQ ID NO: 3.

12. An antibody specifically bound with the LYC3 polypeptide of Claim 4.

13. A nucleotide molecule wherein it is the antisense sequence of the DNA molecule of Claim 1.

14. A probe wherein it comprises about 8-100 consecutive nucleotides of the DNA molecule of Claim 1.

ABSTRACT

The invention relates to a novel member LYC3 of lysozyme gene family. The invention provides the cDNA sequence encoding for the novel lysozyme, the polypeptide encoded by the sequence, as well as the method for producing said novel human lysozyme utilizing recombinant technology. The invention also provides the use of the novel human lysozyme.

```
                  10         20         30         40         50         60
LYC3        MLLALVCLLSCLLPSSEAKLYGRCELARVLHDFGLDGYRGYSLADWVCLAYFTSGFNAAA
            :   ..  :  :  . ..:.. ::::::.:. .::::::.: :::.::::: . ::.:. :
gi|1790947 MRALIILGLVLLSVTVQGKIPERCELARTLKKLGLDGYKGVSLANWVCLAKWESGYNTEA
                  10         20         30         40         50         60

                   70         80         90        100        110
LYC3        LDYE-ADGSTNNGIFQINSRRWCSN-LTPNVPNVCRMYCSDLLNPNLKDTVICAMKITQE
             .:. .: ::. :::::::: ::.:  ::.. ..:.. :: ::. :. :.: :: .....
gi|1790947 TNYNPGDESTDYGIFQINSRYWCNNGKTPGAVDACHISCSALLQNNIADAVACAKRVVSD
                70         80         90        100        110        120

          120        130        140
LYC3        PQGLGYWEAWRHHCQGKDLTEWVDGCDF
            :::.  : :::.:::.::....: ::
gi|1790947 PQGIRAWVAWRNHCQNKDVSQYVKGCGV
                  130        140
```

Fig. 1A

```
                  10         20         30         40         50
LYC3        MLLALVCLLSCLLP-SSEAKLYGRCELARVLHDFGLDGYRGYSLADWVCLAYFTSGFNAA
            :   :. .: :.:: .. .:.:::::::: ... .:::.:::::::.::: : : :.::..
sp|00702 MRSLLILVL-CFLPLAAPGKVYGRCELAAAMKRMGLDNYRGYSLGNWVCAAKFESNFNTG
                  10         20         30         40         50

         60         70         80         90        100        110
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            : . ..:::::. :::.::::: ::..  ::.  :.:.. :: ::. ..  .: :: ::...
sp|00702 ATNRNTDGSTDYGILQINSRWWCNDGRTPGSKNLCHIPCSALLSSDITASVNCAKKIVSD
         60         70         80         90        100        110

         120        130        140
LYC3        PQGLGYWEAWRHHCQGKDLTEWVDGCDF
             .:.. : :::.::.: :.. :. :: .
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         120        130        140
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Fig. 1B

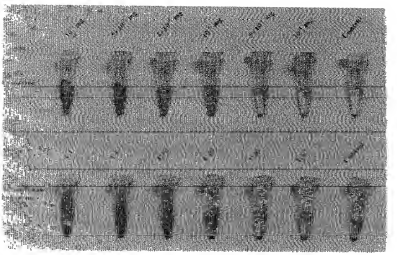

Fig. 2

BAKER & BOTTS, L.L.P.
FILE NO.: RBH A 34052 PCT

COMBINED DECLARATION AND POWER OF ATTORNEY

(Original, Design, National Stage of PCT, Divisional, Continuation or C-I-P Application)

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

This declaration is of the following type:

- [] original
- [] design
- [X] national stage of PCT.
- [] divisional
- [] continuation
- [] continuation-in-part (C-I-P)

the specification of which: *(complete (a), (b), or (c))*

(a) [ ] is attached hereto.
(b) [ ] was filed on as Application Serial No. and was amended on *(if applicable).* 30 Aug. 1999
(c) [X] was described and claimed in PCT International Application No. PCT/CN99/00132 filed on and was amended on *(if applicable).*

Acknowledgement of Review of Papers and Duty of Candor

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of the subject matter claimed in this application in accordance with Title 37, Code of Federal Regulations § 1.56.

[ ] In compliance with this duty there is attached an information disclosure statement. 37 CFR 1.98.

Priority Claim

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT International Application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International Application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application on which priority is claimed

(complete (d) or (e))

(d) [ ] no such applications have been filed.
(e) [X] such applications have been filed as follows:

PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION			
COUNTRY APPLICATION NO.	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
CN 98111041.X	**31.08.1998**		[X] YES NO [ ]
			[ ] YES NO [ ]
			[ ] YES NO [ ]
ALL FOREIGN APPLICATION(S), IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION			
			[ ] YES NO [ ]
			[ ] YES NO [ ]
			[ ] YES NO [ ]

Claim for Benefit of Prior U.S. Provisional Application(s)

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

Provisional Application Number	Filing Date

Claim for Benefit of Earlier U.S./PCT Application(s) under 35 U.S.C. 120

(complete this part only if this is a divisional, continuation or C-I-P application)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose information as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

(Application Serial No.) (Filing Date) (Status) (patented, pending, abandoned)

(Application Serial No.) (Filing Date) (Status) (patented, pending, abandoned)

Power of Attorney

As a named inventor, I hereby appoint Dana M. Raymond, Reg. No. 18,540; Frederick C. Carver, Reg. No. 17,021; Francis J. Hone, Reg. No. 18,662; Joseph D. Garon, Reg. No. 20,420; Arthur S. Tenser, Reg. No. 18,839; Ronald B. Hildreth, Reg. No. 19,498; Thomas R. Nesbitt, Jr., Reg. No. 22,075; Robert Neuner, Reg. No. 24,316; Richard G. Berkley, Reg. No. 25,465; Richard S. Clark, Reg. No. 26,154; Bradley B. Geist, Reg. No. 27,551; James J. Maune, Reg. No. 26,946; John D. Murnane, Reg. No. 29,836, Henry Tang, Reg. No. 29,705, Robert C. Scheinfeld, Reg. No. 31,300, John A. Fogarty, Jr., Reg. No. 22,348, Louis S. Sorell, Reg. No. 32,439 and Rochelle K. Seide Reg. No. 32,300 of the firm of BAKER & BOTTS, L.L.P., with offices at 30 Rockefeller Plaza, New York, New York 10112, as attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

SEND CORRESPONDENCE TO:	DIRECT TELEPHONE CALLS TO:
BAKER & BOTTS, L.L.P. 30 ROCKEFELLER PLAZA, NEW YORK, N.Y. 10112 CUSTOMER NUMBER: 21003	BAKER & BOTTS, L.L.P. (212) 705-5000

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section

1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF SOLE OR FIRST INVENTOR	LAST NAME: Yu	FIRST NAME: Long	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY: Shanghai	STATE or FOREIGN COUNTRY: China CNX	COUNTRY OF CITIZENSHIP: China	
POST OFFICE ADDRESS	POST OFFICE ADDRESS: Institute of Genetics, Fudan University	CITY: Shanghai	STATE or COUNTRY: China	ZIP CODE: 200433
DATE: 2/16/01	SIGNATURE OF INVENTOR: Yulong			
FULL NAME OF SECOND JOINT INVENTOR, IF ANY	LAST NAME: Fu	FIRST NAME: Qiang	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY: Shanghai	STATE or FOREIGN COUNTRY: China CNX	COUNTRY OF CITIZENSHIP: China	
POST OFFICE ADDRESS	POST OFFICE ADDRESS: Institute of Genetics, Fudan University	CITY: Shanghai	STATE or COUNTRY: China	ZIP CODE: 200433
DATE: 2/16/01	SIGNATURE OF INVENTOR: Qiang Fu			
FULL NAME OF THIRD JOINT INVENTOR, IF ANY	LAST NAME: Zhao	FIRST NAME: Yong	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY: Shanghai	STATE or FOREIGN COUNTRY: China CNX	COUNTRY OF CITIZENSHIP: China	
POST OFFICE ADDRESS	POST OFFICE ADDRESS: Institute of Genetics, Fudan University	CITY: Shanghai	STATE or COUNTRY: China	ZIP CODE: 200433
DATE: 2/16/01	SIGNATURE OF INVENTOR: Yong Zhao			
FULL NAME OF FOURTH JOINT INVENTOR, IF ANY	LAST NAME: Zhang	FIRST NAME: Honglai	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY: Shanghai	STATE or FOREIGN COUNTRY: China CNX	COUNTRY OF CITIZENSHIP: China	
POST OFFICE ADDRESS	POST OFFICE ADDRESS: Institute of Genetics, Fudan University	CITY: Shanghai	STATE or COUNTRY: China	ZIP CODE: 200433
DATE: 2/16/01	SIGNATURE OF INVENTOR: Honglai Zhang			
FULL NAME OF FIFTH JOINT INVENTOR, IF ANY	LAST NAME: Bi	FIRST NAME: Anding	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY: Shanghai	STATE or FOREIGN COUNTRY: China CNX	COUNTRY OF CITIZENSHIP: China	
POST OFFICE ADDRESS	POST OFFICE ADDRESS: Institute of Genetics, Fudan University	CITY: Shanghai	STATE or COUNTRY: China	ZIP CODE: 200433
DATE: 2/16/01	SIGNATURE OF INVENTOR: Bi Anding			

Check proper box(es) for any added page(s) forming a part of this declaration

[ ] Signature for ninth and subsequent joint inventors. Number of pages added _____.

[ ] Signature by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. Number of pages added _____.

[ ] Signature for inventor who refuses to sign, or cannot be reached, by person authorized under 37 CFR 1.47. Number of pages added _____.

A 34254 8-27

Attorney Docket No. _
Baker Botts L.L.P.

Applicant or Patentee: _____
Serial or Patent No.: _ Filed or Issued: _____
For: _____

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(b)) - INDEPENDENT INVENTOR

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled __described in

[ ] the specification filed herewith
[ ] Application Serial No. __, filed __.
[ ] Patent No. __, issued __.

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below.

[ ] no such person, concern, or organization
[ ] persons, concerns or organizations listed below*

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities (37 CFR 1.27)

NAME Yu , Long

ADDRESS Handan Road 220, Institute of Genetics, Fudan University, Shanghai 200433. PRC

[X] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

NAME _____

ADDRESS _____

[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Yu , Long	
NAME OF INVENTOR	NAME OF INVENTOR
Yulong	
Signature of Inventor	Signature of Inventor
Feb.18, 2001	
Date	Date

<110> Yu, Long
Fu, Qiang
Zhao, Yong
Zhang, Honglai
Bi, Anding

<120> A NOVEL HUMAN LYSOZYME GENE, ITS ENCODED POLYPEPTIDE AND THE METHOD FOR PREPARING THEM

<130> A34054-PCT-USA 072975.0110

<140> 09/786,024
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Ala Asp Trp Val Cys Leu Ala Tyr Phe Thr Ser Gly Phe Asn Ala Ala
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Ala Leu Asp Tyr Glu Ala Asp Gly Ser Thr Asn Asn Gly Ile Phe Gln
60                  65                  70                  75

atc aac agc cgg agg tgg tgc agc aac ctc acc ccg aac gtc ccc aac   353
Ile Asn Ser Arg Arg Trp Cys Ser Asn Leu Thr Pro Asn Val Pro Asn
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gtg tgc cgg atg tac tgc tca gat ttg ttg aat cct aat ctc aag gat   401
Val Cys Arg Met Tyr Cys Ser Asp Leu Leu Asn Pro Asn Leu Lys Asp
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acc gtt atc tgt gcc atg aag ata acc caa gag cct cag ggt ctg ggt   449
Thr Val Ile Cys Ala Met Lys Ile Thr Gln Glu Pro Gln Gly Leu Gly
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tac tgg gag gcc tgg agg cat cac tgc cag gga aaa gac ctc act gaa   497
Tyr Trp Glu Ala Trp Arg His His Cys Gln Gly Lys Asp Leu Thr Glu
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Phe Gly Leu Asp Gly Tyr Arg Gly Tyr Ser Leu Ala Asp Trp Val Cys
        35                  40                  45
Leu Ala Tyr Phe Thr Ser Gly Phe Asn Ala Ala Ala Leu Asp Tyr Glu
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Cys Ser Asp Leu Leu Asn Pro Asn Leu Lys Asp Thr Val Ile Cys Ala
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35 40 45
Leu Ala Lys Trp Glu Ser Gly Tyr Asn Thr Glu Ala Thr Asn Tyr Asn
50 55 60
Pro Gly Asp Glu Ser Thr Asp Tyr Gly Ile Phe Gln Ile Asn Ser Arg
65 70 75 80
Tyr Trp Cys Asn Asn Gly Lys Thr Pro Gly Ala Val Asp Ala Cys His
85 90 95
Ile Ser Cys Ser Ala Leu Leu Gln Asn Asn Ile Ala Asp Ala Val Ala
100 105 110
Cys Ala Lys Arg Val Val Ser Asp Pro Gln Gly Ile Arg Ala Trp Val
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Ala Ala Lys Phe Glu Ser Asn Phe Asn Thr Gly Ala Thr Asn Arg Asn
50 55 60
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Trp Cys Asn Asp Gly Arg Thr Pro Gly Ser Lys Asn Leu Cys His Ile
85 90 95
Pro Cys Ser Ala Leu Leu Ser Ser Asp Ile Thr Ala Ser Val Asn Cys
100 105 110
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115 120 125
Trp Arg Lys His Cys Lys Gly Thr Asp Val Asn Val Trp Ile Arg Gly
130 135 140
Cys Arg Leu
145

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☐ Page(s)_____ of _____ were not present for scanning. (Document title)

☑ ***Scanned copy is best available.*** Figure 2 is dark.